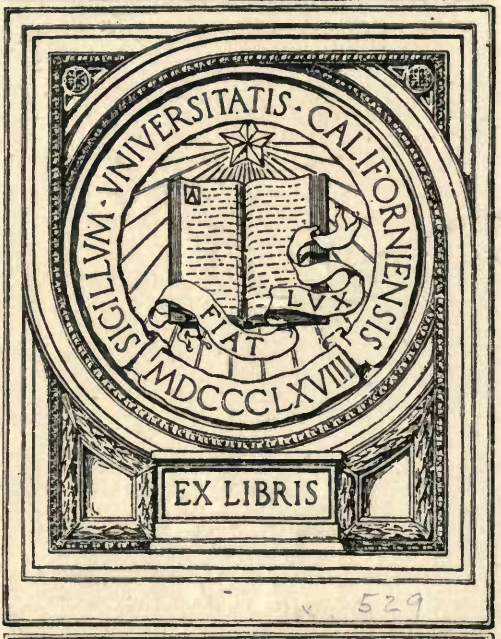

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SOME FACTORS INFLUENCING THE QUANTITATIVE DETERMINATION OF GLIADIN

BY
J. E. GREAVES

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SOME FACTORS INFLUENCING THE QUANTITATIVE DETERMINATION OF GLIADIN*

BY

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(From the Chemical Laboratory of the Utah Experiment Station and the Rudolph Spreckels Physiological Laboratory of the University of California)

CONTENTS

* Presented in partial fulfillment of the requirement for the degree of Doctor of Philosophy in the University of California. The author wishes to express his thanks to Dr. Stewart, of the Utah Experiment Station, for the samples of flour and the polariscope used in this work; also to Dr. Robertson, of the University of California, for the many kind suggestions received from him in the work.

INTRODUCTION

In testing the chemical composition of flours in work which is being carried on by a number of stations on the milling qualities of wheat, it is necessary to make each year a great number of gliadin determinations. The method[72] which has been used by the author gives comparative results if followed closely; but where the number of samples is large, the time required to make the determinations is too great; and the polariscope method given by Snyder[68] has not been applicable in most cases. Furthermore, no systematic work has been done to correlate the results obtained under varying conditions with these two methods. Therefore, this work is undertaken for two reasons: first, to so modify the polariscope method that it may be used with flours from all wheat; and second, to find the relationship existing between the polariscope method and the Kjeldahl method, together with some of the factors which influence the results obtained by these methods.

HISTORICAL RESUMÉ

A thorough review of the literature has been made with the intention of noting the main properties of gliadin and examining the various methods which have been proposed by different investigators, and in some cases the objections which have been raised to them.

Following is a brief review of the literature on the subject up to the present time:

Einhof[13] (1805) was the first to state that alcohol extracted from wheat flour a protein substance, but he considered it to be the same as gluten.

Taddei[73] (1820), an Italian chemist, separated wheat gluten into two parts: one soluble in alcohol to which he gave the name gliadin, the other insoluble and called by him zymon. He also studied the action of water, alcohol, acids, and alkalies on these substances.

Boussingault[7] (1837) was of the opinion that the entire gluten is soluble in alcohol.

Liebig[38] (1841) named the part of gluten which is soluble in alcohol, plant gelatin, and Mulder[46] (1844) considered this plant gelatin to be a compound of a protein, and sulphur free from phosphorus.

Günsberg[24] (1862) considered gliadin to be a mixture of two substances: one soluble in boiling water, the other soluble in alcohol. The former yielded 17.78 per cent nitrogen, the latter 14.10 per cent.

Ritthausen[64] (1872) published a volume on the properties of the wheat kernel in which he gave the ultimate analysis together with all the known properties of gliadin, or, as he calls it, plant gelatin. In this work, he considers the proteins of gluten as being four in number, one insoluble in alcohol, gluten-casein, and three soluble in alcohol, gliadin, gluten-fibrin and mucedin.

Wigner[78] (1878) found 17.7 per cent of the total nitrogen present in wheat in such a form that it is not precipitated by carbolic acid.

Weyl and Bischoff[77] (1880) held that gluten does not exist as such in flour, but is due to the action of some ferment on the vegetable myosin of flour, for they found that flour which had been extracted with a 15 per cent sodium chloride solution yielded no gluten. This was also the case when the flour had been heated at 60° C. for several hours. It was thought, however, that the failure to obtain gluten in the latter case was due to the coagulation of the myosin.

Martin[44] (1886) held that gluten does not exist as such in flour, but is due to a reaction between the protein and water. This reaction may be brought about by the action of a ferment. He found that gliadin, which he calls phytalbumose, is soluble in alcohol and in hot water. The main properties of the compound are given and a scheme of the relationship existing between gluten and its supposed precursors.

Johannsen[30] (1889) opposed the theory that gluten is formed by a special ferment, and cited facts which he claimed are against such a theory.

Osborne and Voorhees[50] (1892) described very minutely the properties of gliadin as follows: "Soluble in dilute alcohol and

forming about 4.25 per cent of the seed. It is soluble in distilled water to opolescent solution which is precipitated by adding a very little sodium chloride. It is completely insoluble in absolute alcohol, but slightly soluble in 90 per cent alcohol. It is precipitated from these solutions on adding either much water or strong alcohol, especially in the presence of much salts. Soluble in very dilute acids and alkalis, and precipitated from these solutions by neutralization, unchanged in properties and composition. This proteid is one on which the formation of gluten depends.'' They[50a] also (1893) showed that the formation of gluten is not due to the action of a ferment.

Balland[55] (1893) considered the view that gluten pre-exists in flour to be correct, for he obtained the same amount of gluten when prepared with water of different temperatures, 2° C., 15° C., and 60° C. Furthermore, the yield of gluten was equally great when the flour had been previously treated with a disinfectant.

Fleurent[14] (1894) decomposed the vegetable proteins by means of barium hydroxide and determined the decomposition products thus yielded, from which he concluded that vegetable and animal proteins are different.

O'Brien[49] (1895) studied the properties of gluten and used various strengths of alcohol to extract the gliadin. He concluded from his work, together with a critical examination of that of previous investigators, that there is one parent substance in flour which yields gluten, and that glutenin and gliadin are derived from the same parent substance, the former being a hydrated form of the latter.

Kjeldahl[31] (1896) found the alcohol-soluble portion of flour to contain about 52 per cent carbon, 17.25 per cent nitrogen, and having a specific rotation of —92. These factors he found to be practically constant, even when obtained from a great variety of wheats from different localities, and grown during different seasons. From this he concluded that there is only one alcohol-soluble protein in flour. He determined its specific rotation in dilute acids, alkalies, phenol, and acetic acid. According to him, gliadin reaches its maximum solubility in 55 per cent alcohol and it is nearly insoluble in 20 and 90 per cent alcohol.

Ritthausen[65] (1896) by collecting and studying data from various sources concluded that there is 17.6 per cent nitrogen in the protein of cereals, hence in their calculation the factor 5.7 should be used in place of 6.25, as had been previously used.

Fleurent[15] (1896) believed that flour contained only one protein soluble in alcohol; for which he proposed a method for its quantitative determination.[21] This consisted in treating the gluten with alcohol containing potassium hydroxide, thus dissolving the gliadin and part of the glutenin, the latter being precipitated later by means of carbonic acid, the gliadin determined by filtering and evaporating a portion of the filtrate to dryness. The residue thus obtained, when corrected for carbonate, was considered as gliadin. Gluten was found by this method to contain from 60 to 80 per cent gliadin, and according to the same author, the ratio of gliadin to glutenin determines the bread-making qualities of flour. Later Guthrie[25] (1896) stated that the strength of flour depends upon the proportion of gliadin to glutenin, strong flours being those comparatively rich in glutenin.

Teller[74] (1896) devised a method for the determination of gliadin in wheat flour and determined the quantity in various flours. He claimed[75] (1897) that the proteosé of Osborne and Voorhees was gliadin. Osborne,[51] (1897) however, showed that he had not mistaken that part of gliadin which is soluble in a salt solution for a proteose, but that he had actually prepared from wheat flour a proteose.

Girard[22] (1897) reported the methods used in testing flours and gave the composition of a number of different flours.

Fleurent[16] (1898) found a relationship existing between the value of flour and the composition of its gluten, and that a good flour should contain one part of glutenin to three parts of gliadin. In order to make poor flours conform to this standard, he states that it is sometimes the practice to add bean flour in order to increase the amount of glutenin present.

Morishima[45] (1898) agreed with Osborne and Voorhees that the alcohol-soluble part of wheat gluten contains but one protein (gliadin). He considers gluten of wheat flour to contain only one protein to which he gave the name "artoline." This,

according to the same author, contains only carbon, hydrogen, oxygen, and nitrogen. The substances gliadin, mucedin, gluten-fibrin, glutenin and gluten-casein, are believed to be mixtures of artoline and a phosphorus body in union with a base.

Ritthausen[66] (1899) reviewed the work which had been done on the proteins of wheat, and criticized the work of Morishima.

Guess[23] (1900) modified the method of determining gliadin and studied the relationship of gliadin to the bread-yielding qualities of flours.

Kossel and Kutscher[33] (1901) found that on hydrolysis gliadin yielded no lysin, and they held the view that there are three alcohol-soluble proteins. These they separated by fractionation from alcohol and determine their decomposition products.

Hamann[26] (1901) devised a method for determining gliadin in which he used 70 per cent alcohol containing one per cent acetic acid for the extraction of gliadin.

Fleurent[17] (1901) proposed a method for determining the per cent of gliadin in flour by means of an instrument called the densimeter, which is based upon the relationship existing between the specific gravity and the amount of gliadin which the alcoholic extract contains. Later[18] he (1901) found that this method when used in testing flour from hard wheats gave results which are much too low even after a correction had been made for the soluble sugars.

Manget[40] (1902) modified the original Fleurent method mainly to provide for the amount of water which is extracted from the moist gluten by the alcohol.

Osborne[52] (1902) found gliadin to contain 1.027 per cent of total sulphur, and 0.619 per cent of loosely combined sulphur. From these determinations he calculated its probable molecular weight.

Kossel and Kutscher[33] (1902) made determinations of the diamino acids in preparations believed to represent the three proteins of wheat soluble in alcohol,—mucedin, gliadin, and gluten-fibren, which Ritthausen had described as the alcohol soluble constituents of wheat gluten.

Osborne and Harris[53] (1903) tested gliadin for the carbohydrate group, and while it gave the Molisch's furfuraldehyde

reaction, there was found no other proof of the presence of the carbohydrate group in the gliadin molecule. They[54] found the rotating power of gliadin to be —92.28, but there was nothing in their work which pointed to the existence of more than one protein of gluten soluble in alcohol. The same authors[55] made an extensive study of the various forms of nitrogen yielded on decomposing gliadin.

Kosutany[34] (1903) concluded from his work that gliadin does not exist as such in flour, but is a hydrated form of gluten. He cites in support of his theory the fact that when a clear solution of gliadin is allowed to stand for some days in 70 per cent alcohol, a precipitate is formed; this he thinks to be due to the gliadin splitting off water and forming glutenin. Later[35] he showed that the per cent of nitrogen extracted by 70 per cent alcohol from the flour is greater than that extracted from the gluten.

Nasmith[48] (1903), however, is of the opinion that the gliadin and glutenin are not derived from the same parent substances, for the composition and physical properties of the two are found to be very different. He found further, that gliadin invariably gives the reaction for organic iron and phosphorus, and that it is precipitated by an excess of acid.

Fleurent[19] (1903) gave a modified method of determining gliadin of wheat flours by means of the gliadimetre together with its limits of accuracy and claimed that close relationship existed between the results thus obtained and the bread making qualities of flour.

Kutscher[36] (1903) determined the amount of tyrosine and glutaminic acid yielded by gliadin on decomposing it with sulphuric acid. It was found to yield 2.09 per cent of tyrosine and 18.54 per cent of glutaminic acid.

Using the work of Osborne and Voorhees as a basis, Snyder[68] (1904) proposed a method for the determination of gliadin in wheat flour by means of the polariscope. This method he found to give results that agree very closely with those obtained from the determination of nitrogen by the Kjeldahl method. The sugars and non-protein substances dissolved, he found to be so small as to be negligible. However, unless special

precautions were taken, he was troubled with a cloudy filtrate.

Konig and Rintelin[32] (1904) reported their investigations as being in harmony with those of Ritthausen. They found what they thought to be three distinct proteins of wheat gluten which were soluble in alcohol of 60 to 70 per cent, and one of these, gluten-fibrin, was dissolved by stronger alcohol, 88 to 90 per cent; and one of the others, mucedin, was soluble in alcohol of from 30 to 40 per cent. They gave the following as the elementary composition of these substances:

	C.	H.	N.	S.	O.
Glutin Fibrin	55.30	8.17	16.86	1.07	19.73
Gliadin	52.70	7.62	17.77	0.95	20.96
Mucedin	53.33	8.07	16.83	0.78	20.99

Chamberlain[8] (1904) studied the Fleurent-Manget method and found that it gave results which are too high. He found that part of the gliadin was soluble in a salt solution. From his work he suggested a method based on the work of Osborne and Snyder.

Osborne and Harris[56] (1904) reviewed the literature of the alcohol-soluble proteins of wheat flour and made determinations of the amount of glutaminic acid yielded by the different preparations of gliadin. They consider that Kutscher's determinations of the glutaminic acid yielded by the various fractions precipitated from alcohol offers no evidence of their being more than one protein of gluten soluble in alcohol, while their own work indicates the presence of only one, and for this protein they hold the name gliadin should be retained.

Snyder[69] (1905) emphasized the value of gliadin determinations as follows: "The gliadin determinations, however, have been very helpful in determining abnormal conditions in the composition of wheats and the results available at the present time indicate that the percentage amounts of gliadin in flour is of more importance than the gliadin-gluten ratio. In comparing different grades of flour milled from the same wheat, differences are observed in the gliadin percentage, the lower grades of flour having a tendency to contain proportionally less gliadin than higher grades." He also expresses the view that gliadin is not a definite chemical compound.

Norton[47] (1905) found it necessary to make a correction for the sugars in the alcoholic extract, when determining the gliadin by means of the polariscope. This he found to be especially true in the case of the Durum wheats, where the correction varied from 0.7 to 1.8 per cent on the sugar scale of a Schmidt and Haensch polariscope.

Abderhalden and Samuely[1] (1905) made quantitative determinations of the various amino-acids yielded by gliadin on decomposition.

Mathewson[42] (1906) found that the specific rotation of gliadin solutions is but little affected by ordinary changes in the gliadin concentration of the alcoholic extract. When an account is taken of the expansion of the solvent due to heat, the temperature at which the reading was made, between the degrees of 20° and 45° C., was found to have but little influence on the rotation. However, he did find that there was a large variation of the specific rotation of the extract depending upon the per cent of alcohol used as a solvent. He made specific gravity determinations of alcoholic solutions of gliadin but found that the variation of the specific gravity with the amount of gliadin in the solution was not sufficient to give any great accuracy in the determination of gliadin by the Fleurent method. Later[43] he found gliadin to be soluble in dilute methyl and propyl alcohol, glacial acetic acid, phenol, paracresol and benzyl alcohol. A white precipitate was obtained by adding ether, acetone, pyridine, benzene, and chloroform to phenol solutions of gliadin. The same was the case on the addition of ethyl, prophyl, or amyl alcohol, but no precipitate was obtained on adding analine, phenylhydrazine, or nitrobenzine. The specific rotation of gliadin solutions in various concentrations of the above solvents and with various concentrations of gliadin solutions were determined.

Osborne and Harris[57] (1906) analyzed twenty-five samples of gliadin and obtained as an average of these analyses 52.72 per cent carbon, 6.86 per cent hydrogen, 17.66 per cent nitrogen, 1.14 per cent sulphur, and 21.62 per cent oxygen. The elementary composition of gliadin and glutenin were nearly the same, but the authors are of the opinion that these are distinct compounds.

Osborne and Clapp[58] studied the decomposition products of various proteins and from their work they concluded that leucosin, glutenin, and gliadin are separate and distinct bodies. The latter was found to be free from glycocoll.

Chamberlain[9] (1906) reviewed briefly the work of previous investigators on the proteins of the wheat kernel. He studied the action of hot and cold alcoholic extraction and also the influence of varying amounts of solvents on the yield of gliadin. Alcohol was found to dissolve other proteins beside gliadin. These he believed to be the albumen and proteose of the flour. He suggested an explanation of the different results obtained by various investigators as being due to the fact that some investigators have worked with the gluten of flour while others have used the flour.

Snyder, Hummel and Shutt[70] (1906) each found that the amount of substance extracted from flour varies with the strength of the alcohol used as a solvent. Forty to 60 per cent alcohol was found to extract the greatest amount of non-gliadin material. Hummel found that practically all of the alcohol-soluble protein is extracted by twenty-four hours extraction.

Marion[41] (1906) proposed a method for the determination of gliadin by means of the polariscope. He extracted ten grams of flour with 50 cc. of 73 per cent alcohol in a closed vessel at from 40 to 45 degrees Centigrade for fifteen minutes. The solution was clarified by means of animal charcoal and polarized.

Abderhalden and Malengrean[2] (1906) determined the percentage of amino-acids yielded by gliadin. Gliadin was found to yield no lysin.

Thatcher[76] (1907) made gliadin determinations of flour in which he used five grams of flour in 250 cc. of 70 per cent alcohol by weight. Attempts were made to use the polariscope method but it was impossible, with the flours under examination, to get filtrates clear enough to polarize.

Shaw[67] (1907) tested the polariscope method with flours from various wheats. He found that it was necessary to make a correction for the non-protein material. This was particularly true in case of wheat meals, where the average difference between the two polariscope readings was 0.21 per cent on the

gliadin scale. In the case of most flours the difference was but 0.04 per cent on the gliadin scale.

Lindet and Ammann[39] (1907) found that the specific rotation of alcoholic extracts of gliadin diminished as the strength of the alcohol became greater than 70 per cent, and the rotation is proportional to the amount of protein dissolved.

They concluded from their work that gliadin is made up of two constituents, one having a specific rotation of $(\alpha)\ \frac{20}{D} = -81.6$, while the other had a specific rotation of $(\alpha)\ \frac{20}{D} = -95$. The rotating power of mixed gliadins as determined on twenty samples was found to vary between —81.6 and —92.7 degrees. The names of Alpha and Beta-gliadin were proposed for the two substances.

Osborne and Clapp[59] (1907) reported a new decomposition product yielded by gliadin on hydrolysis with acid. This substance was thought to be a dipeptide and on further hydrolysis, yielded prolin and phenylalanine.

Osborne[60] (1907) reviewed the work on the proteins of the wheat kernel, and gave a complete summary of all his work on the protein of wheat. In this he gives a concise review of the most important properties of the gliadin together with the other known proteins of wheat.

Abderhalden and Emmerling[3] (1907) allowed *Bacillus mesentericus vulgatus* to act on gliadin for six weeks. At the end of this time they determined the amino-acids yielded. All were present that are yielded on acid hydrolysis but not in as large quantities. This was thought to be due to the comparatively short time that the bacillus had to act upon the substance.

Ladd[37] (1907) recommended the extraction of four grams of flour with 200 cc. of alcohol of 0.90 sp. gr. for fifteen hours, at the end of which time the gliadin was determined by the Kjeldahl method. Where the polariscope was used he found it necessary to make a correction for the non-protein material present. This was done by precipitating the proteins with Millon's reagent and then making a second polarization.

Benedict and Osborne[6] (1907) found that one gram of gliadin on being burned liberated 5738 cal. of heat.

Wood[79] (1908) claimed that his work showed the chemical composition of the gliadin and glutenin of strong and weak flours to be identical.

Mathewson[43a] (1908) found that the amount of gliadin extracted by alcohol varied with the ratio of the alcohol to the flour used in the extraction. He found that drying the flour before extraction rendered the gliadin less soluble. There was no evidence for the theory that glutenin removes gliadin from its alcoholic solution by absorption. He found that a large portion of the protein of flour is soluble in phenol. This, however, is not all gliadin.

Osborne[61] (1909) issued a monograph on the vegetable proteins in general, in which are given the main properties of gliadin. There is also appended a complete bibliography of the work which has been done in this field.

Henriques[28] (1909) experimenting on rats found that gliadin can maintain nitrogen equilibrium and even lead to the storage of nitrogen.

Osborne[62] (1910) in a masterly treatise on the plant proteins gave the main properties of gliadin together with a review of the literature leading up to the present knowledge.

Robertson and Greaves[66a] (1911) made determinations of the refraction indices of gliadin in acetic acid, potassium hydroide, acetone, phenol, ethyl, and propyl alcohol and found the refraction index of the solvent to be increased by the gliadin in every case except with 75 per cent phenol.

EXPERIMENTAL

Properties of Gliadin

Before taking up the results obtained in this work it may be well to consider briefly the properties of Gliadin.

Gliadin is that material in flour which, under the influence of water, forms a sticky medium which binds together the particles of flour, rendering the dough and gluten tough and coherent. The substance, according to Mathewson[43] is soluble in methyl, ethyl, propyl, amyl, and benzyl alcohols; phenyl, paracresol, acetic acid, dilute mineral acids[44] and alkalines. Osborne[50] states that gliadin is slightly soluble in pure water, but less so

in water containing sodium chloride. Teller,[75] however, claims that sodium chloride extracts considerable of the alcohol-soluble proteins from flour. Nasmith[48] also found that gliadin is not entirely insoluble in dilute salt solutions, while Martin[44] gives it as being soluble in boiling water. The solvent which is most often used, however, is dilute ethyl alcohol. In this substance, according to Kjeldahl,[31] it reaches its maximum solubility in fifty-five per cent and decreases in solubility in weaker and stronger solutions, reaching zero in 20 and 90 per cent alcohol. Osborne[60] gives the maximum solubility as being in 70 per cent alcohol and completely insoluble in absolute alcohol. Alcoholic solutions of this substance can be boiled, according to the latter author, without changing its composition or properties. The substance is precipitated from this solvent by the addition of strong alcohol, ether, or salt solutions; also, according to Nasmith,[48] by an excess of acid. The substance is optically active in the above solvents, the degree varying with the solvent, also in most cases with strength of solvent used. This is shown by the following table, which has been taken from the works of various investigators.

Solvent	Specific Rotation	Solvent	Specific Rotation
Methyl alcohol 70%	—95.6[43]	Propyl alcohol 60%	—101.1[43]
Ethyl alcohol 70%	—91.9[43]	Phenol 40%	—130.0[31]
Ethyl alcohol 70%	—92.0[33]	Phenol 70%	—123.1[43]
Ethyl alcohol 70%	α —81.6[39]	Phenol anhydrous	—131.6[43]
Ethyl alcohol 70%	β —95.0[39]	Para cresol	—121.0[43]
Ethyl alcohol 60%	—96.6[43]	Acetic acid anhydrous	— 79.8[43]
Ethyl alcohol 55%	—92.0[31]	Acetic acid 0.1% to 5%	—111.0[31]
Ethyl alcohol 50%	—98.4[43]	Acetic acid anhydrous	— 81.0[31]
		Benzyl alcohol	— 55.7[43]

Chemical Composition

Gliadin contains carbon, hydrogen, oxygen, nitrogen and sulphur. According to Chittenden and Smith[12] it has the following composition. The results given are the average of eight determinations.

Carbon	52.87
Hydrogen	6.99
Nitrogen	15.86
Sulphur	1.17
Oxygen	23.11

These results in the case of carbon, hydrogen and sulphur

are very near the results obtained by Osborne,[57] Nasmith,[48] and Kjeldahl.[31] However, the latter investigators obtain a larger per cent of nitrogen (about 2 per cent more) and a smaller per cent of oxygen (about 2 per cent less). Osborne[52] found that 0.619 per cent of sulphur in gliadin was loosely combined. According to Nasmith[48] it contains in addition to the above element, 0.267 per cent of phosphorus and 0.034 per cent of iron. Morishima[45] is also of the opinion that gliadin contains phosphorus. However, Abderhalden,[4] as may be seen from the following, is of the opinion that pure gliadin does not contain phosphorus as a constituent of its molecule. "It has not yet been decided definitely whether this phosphorus (referring to the phosphorus obtained by the analysis of gliadin) is actually a part of the protein molecule, or only an impurity. The latter assumption seems justified, as the method of preparation is crude, and very little effort has been made to purify them." Osborne and Harris[56] state very emphatically that gliadin does not contain phosphorus.

Considerable work has been done on the determination of the various products yielded by gliadin on decomposition. In the following table is given a condensed summary of the more important work done in this line.

Arginine	2.75[33]	3.40[36]	2.75[36]	3.16[58]
Histidine	1.20[33]	1.70[36]	1.20[36]	0.61[58]
Glycocoll	0.90[1]	0.00[58]		
Alanine	2.70[1]	2.00[58]		
Amimo Valerianic acid	0.33[1]	0.21[58]		
Leucine	6.00[1]	5.61[58]		
Prolin	2.40[1]	7.06[58]		
Phenylalanine	2.60[1]	2.35[58]		
Glutaminic acid	37.50[1]	37.33[58]		
Aspartic acid	1.30[1]	0.58[58]		
Serine	1.12[1]	0.13[58]		
Tyrosine	2.40[1]	1.20[58]		
Tryptophane about	1.48[1]			
Lysine	0.00[1]	0.00[58]		
Ammonia	4.10[36]	5.11[58]		
Cystine	0.45[58]			

The above table shows a difference in the gliadin used by different investigators. This, however, is likely due to impurities.

Gliadin gives all the color tests of the proteins[61] and Osborne and Harris[53] found that it also gave the Molish's furfuraldehyde reaction. But they think this insufficient evidence to conclude that the molecule contains a carbohydrate complex.

Method of Experimentation

The results reported in the following pages were obtained by the analysis of six different flours which were obtained from wheat grown on the Utah Experimental Arid Farms and milled in the station[72] experimental mill. They were so selected as to represent the wheats of high, medium, and low gluten content of both the Durum and common bread varieties as grown under arid conditions. The flours were obtained from New Zealand, White Club, and Gold Coin of the common bread varieties, and Kahla, Black Don, and Adjini of the Durum varieties.

The extraction of the gliadin, unless otherwise stated, was made by placing a weighed portion of flour together with a measured amount of alcohol into 200 c.c. wide mouth bottles provided with ground glass stoppers. By the use of bottles with ground stoppers the evaporation during extraction is reduced to a minimum and they furnish convenient receptacles in which the solution can be shaken at intervals during extraction. All the results reported are calculated to dry basis and are the average of three or more analyses which in nitrogen determinations agreed to within .025 per cent, and the polariscope determinations within .1 on the sugar scale of a Schmidt and Haensch polariscope, so that analytical errors have been reduced to a minimum.

Preparation of a Clear Filtrate

One of the great objections which has been raised against the polariscope method for determining gliadin, is the fact that the filtrate as obtained, especially from high gluten flours, is cloudy, and for this reason the polarization cannot be made with any degree of accuracy. If the suspended particles which cause the cloudy filtrate be insoluble proteins, as they sometimes are, an

objection can be raised against the Kjeldahl method for determining gliadin, as these will vitiate the results thus obtained. For this reason in both methods of determining gliadin a clear filtrate is of the utmost importance.

Thatcher[76] found it impossible to use the polariscope method on flour obtained from the Washington wheats. They were low in gluten and high in carbohydrates and gave cloudy filtrates. Snyder[68] found that soft wheats frequently gave a filtrate which was cloudy and became more cloudy on standing. The same difficulty was experienced by Shaw.[67] But the last two investigators were able to overcome this difficulty by decreasing the agitation of the samples during extraction.

In order to determine whether this method would give clear filtrates with the flours being examined, tests were made on the six samples of flour described above in which the shaking of the samples during extraction was gradually reduced to such an extent that the solutions could be filtered through double quantitative filters and thus give a clear filtrate. But it was found that by so doing the total nitrogen as determined by the Kjeldahl method had decreased and there was not the agreement between duplicates that was obtained when the samples were thoroughly shaken during extraction; thus showing that this means of obtaining a clear filtrate is not applicable with these flours.

Teller[75] states that he obtained clear solutions of gliadin by filtering through a layer of animal charcoal, while Marion[41] in the polariscope method as outlined by him recommends the stirring of the solution at the end of the extraction period with .8 of a gram of animal charcoal for from one to two minutes before filtering. Both of these methods were tried on the flours under examination, and it was found under these conditions clear filtrates could be obtained, but the animal charcoal in carrying down with it the suspended coloring material of the solution had also carried down some of the gliadin, and this in varying amounts, as was shown by the lack of agreement between duplicate determinations made by the Kjeldahl method.

Harris[27] found that some proteins passed through a porcelain filter unchanged in chemical composition. In a great majority of cases, however, he found the filtered solution to be

more dilute than the unfiltered. Hugounenq[29] found that different proteins passed through unglazed porcelain filters at different rates, some being held back so tenaciously that they could not be washed through with water. These facts gave but little hope of success by this method of filtration; nevertheless, solutions were prepared by filtering through the Chamberlain-Pasteur filter and in this manner clear solutions were obtained; but here again, there was no agreement between the duplicates and it appeared as if part of the gliadin had been held back by the filter.

The method which was finally adopted, after trying various others, was to filter the solutions through asbestos. Filters of this were prepared as follows: a small amount of dry shredded asbestos was placed in the bottom of a Gooch crucible and a pad of about one-half inch made by pouring suspended asbestos upon this. This was washed with alcohol dried, and the solution filtered through it. This gave a rapid method of filtering, and evaporation did not affect to any appreciable extent the concentration of the solution. Clear filtrates were obtained from all the flours by this method and duplicates agreed very closely, thus showing that the filter had no retarding influence on the nitrogenous substances. In order to reduce the color of the solutions, which was very dark from some of the flours, and in this manner to increase the accuracy of the polariscope reading, one-half the amount of flour recommended by Snyder was extracted with 100 cc. of alcohol (100 c.c. of alcohol to 7.985 grams of flour in place of 15.97 grams). The effect of this upon the accuracy of the method in general will be considered later. Solutions prepared as above described and placed in well-stoppered flasks may be kept for several hours without developing the cloudy appearance referred to by Thatcher and Snyder, providing the temperature does not fall to 10° C.

Influences of Ratio of Alcohol to Flour on Gliadin Extracted

These determinations were made by extracting 15.97, 7.985, 3.9925, and 1.9962 grams of each of the flours with 100 c.c. of 70 per cent alcohol by volume, and 15.97, 7.985, and 2 grams

with 74 per cent alcohol for forty-eight hours with occasional thorough shaking. At the end of this time the solutions were filtered as described above and placed immediately into 200 c.c. bottles provided with ground glass stoppers. Twenty c.c. of this solution were used for nitrogen determinations by placing into Kjeldahl digestion flasks with 5 c.c. of concentrated sulphuric acid and the alcohol distilled off, after which 15 c.c. more of concentrated sulphuric acid and .5 gram of mercuric oxide were added and the digestion completed. It was found that care should be taken to drive off as much as possible of the alcohol before the second addition of sulphuric acid, otherwise there is considerable frothing and it requires a longer digestion before a colorless solution is obtained. The total nitrogen was determined as outlined by the Association of Official Agricultural Chemists.[11] The remaining solution was used for polarization. In this a 200 mm. tube was used and an average taken of from six to eight readings.

Table I shows the per cent of nitrogen extracted by 70 and 74 per cent alcohol with varying proportions of flour to alcohol.

Table I.—Per Cent of Nitrogen Extracted from Flours by Means of 70 and 74 Per Cent Alcohol by Volume with Different Ratio of Alcohol to Flour

Grams of Flour to	New Zealand	White Club	Gold Coin	Black Don	Kahla	Adjini	Average
100 c.c. 70% alcohol and 15.97 g. flour	1.450	1.289	1.495	1.618	1.652	1.506	1.502
100 c.c. 70% alcohol and 7.985 g. flour	1.470	1.333	1.508	1.731	1.675	1.592	1.552
Difference	0.020	0.044	0.013	0.113	0.023	0.086	0.050
100 c.c. 70% alcohol and 3.9926 g. flour	1.544	1.397	1.539	1.701	1.767	1.616	1.593
Greater + less — than with charge 7.985	0.074	0.064	0.031	—0.030	0.092	0.024	0.041
100 c.c. 70% alcohol and 1.9963 g. flour	1.553	1.347	1.557	1.748	1.822	1.595	1.604
Greater than with charge of 7.985 g.	0.083	0.014	0.049	0.017	0.147	0.003	0.052
100 c.c. 74% alcohol and 15.97 g. flour	1.179	1.030	1.196	1.304	1.405	1.259	1.229
100 c.c. 74% alcohol and 7.985 g. flour	1.278	1.106	1.291	1.329	1.448	1.289	1.290
Difference	0.099	0.076	0.095	0.025	0.043	0.030	0.061
100 c.c. 74% alcohol and 2 grams of flour	1.282	1.094	1.267	1.336	1.419	1.270	1.278
Greater + less — than with charge of 7.985	0.004	—0.008	—0.024	0.005	—0.029	—.019	—.012

An examination of the above table shows that the total alcohol-soluble protein is not obtained with either 70 or 74 per cent alcohol when 15.97 grams of flour are extracted with 100 c.c. of alcohol, even after the expiration of forty-eight hours, for in the above determinations the solvent was allowed to act for that length of time.

It may be seen that the greater amount extracted by the alcohol when a small amount of flour was used in proportion to the alcohol is not a constant, but appears to vary with the flour and strength of alcohol; reaching the great difference of .113 per cent nitrogen with Black Don and only .013 per cent with Gold Coin. The average difference of the two strengths of alcohol, 70 and 74 per cent are nearly the same, being .051 and .061 per cent respectively. With the 70 per cent alcohol there is a greater amount of nitrogen obtained when the ratio is 3.9926 grams of flour to 100 c.c. of alcohol, and the amount is still greater when the ratio is 1.9963 grams of flour to 100 c.c. of alcohol. Where 74 per cent alcohol was used as a solvent the nitrogen obtained is about the same whether 100 c.c. of alcohol was used to extract 7.985 grams or 2 grams of flour. Where there is a difference it is within experimental error.

These results, especially as obtained with 70 per cent alcohol, are in keeping with those reported by Chamberlain,[9] who found that in gliadin determinations relatively large amounts of solvent should be used in connection with relatively small amounts of solute. And he recommends that not over 2 grams of flour be used to 100 c.c. of 70 per cent alcohol.

In table II is given the ratio of the per cent alcohol-soluble protein nitrogen as obtained by the Kjeldahl method, divided by the polariscope reading on the sugar scale of a Schmidt and Haensch polariscope. The polarizations were made in 100, 200, and 400 mm. tubes with the charge of 15.97, 7.985, and 3.9925 grams respectively.

Table II.—The Ratio of Per Cent Alcohol-Soluble Nitrogen to Polariscope Reading with Different Charges of Flour in 70 and 74 Per Cent Alcohol by Volume

Grams of Flour to 100 cc. of alcohol	New Zealand	White Club	Gold Coin	Black Don	Kahla	Adjini	Average
15.97 g. of flour to 100 c.c. 70% alcohol	.50	.53	.50	.55	.52	.56	.526
7.985 g. of flour to 100 c.c. 70% alcohol	.51	.53	.50	.56	.51	.54	.525
3.9925 g. of flour to 100 c.c. 70% alcohol	.52	.53	.51	.55	.52	.54	.528
15.97 g. of flour to 100 c.c. 74% alcohol	.48	.44	.45	.47	.46	.48	.463
7.985 g. of flour to 100 c.c. 74% alcohol	.47	.45	.46	.47	.47	.47	.465

An examination of these results show that the ratio of alcohol-soluble protein to polariscope reading varies directly with the concentration of the solution. This is in accord with the results obtained by Mathewson,[42] who found that the specific rotation of carefully prepared gliadin in 70 per cent alcohol is but little affected by ordinary changes in the gliadin concentration of the alcoholic extracts. In the above results, however, the hard wheats show a slightly higher average ratio than do the soft wheats. This may be due to a higher per cent of optically active sugars in the hard wheats.

Inasmuch as the ratio of per cent nitrogen to polariscope reading is a constant* with varying ratios of alcohol to flour, and a greater amount of nitrogen is extracted when the ratio is 7.985 grams of flour to 100 c.c. of alcohol than when it is 15.97 grams of flour to 100 c.c. of alcohol, the former ratio has been used in the following work. That this does not decrease the accuracy of the polariscope method is shown by the following consideration. The dilute solutions are less colored than the more concentrated, and for this reason the error in the polariscope reading is less in the dilute solution than the more concentrated and darker one. An error of .1 on the sugar scale of a

* I.e., in this work within experimental error. That the specific rotation of a great number of organic compounds does vary with the concentration of the solution is a well established fact. Cf. Landolt, Rotation of Organic Substances, Trans. by Long, p. 169. That gliadin belongs to this class is quite likely from the work of Lindet and Ammann (Ann. Inst. Nat. Agron. 1907, pp. 233-243). But from the work reported in these pages the difference would not appear to be of sufficient magnitude to materially affect determinations made by the polariscope method.

Schmidt and Haensch polariscope (and solutions prepared as described above and polarized with care the error need never exceed this amount), would be equal to .046 per cent nitrogen in 74 per cent alcohol; while with the method as recommended by Snyder using the factor .2 as obtained by him, there would be an error of .02 per cent nitrogen. This would leave the results .026 per cent nitrogen in favor of the ratio 15.97 grams of flour to 100 c.c. of alcohol. But as was shown above, when this ratio is used the nitrogen extracted is .061 per cent, less than when the ratio 7.985 grams of flour to 100 c.c. of alcohol is used. This gives .035 per cent nitrogen in favor of the latter. With some flours which give a fairly colorless solution on extraction, this error may be further reduced by using a 400 m.m. tube in the polarization.

This, however, has not been found advisable with these high gluten flours as the darker color of the column of liquid decreases in too great a measure the accuracy of the polariscope reading.

Influence of Duration of Extraction on Yield of Nitrogen

This was determined by extracting 7.985 grams of each of the flours with 70 and 74 per cent alcohol for 24 and 48 hours. The results obtained are given in table III.

Table III.—Per Cent Nitrogen Extracted and Ratio of Polariscope Reading to Per Cent Nitrogen for Each of the Flours After Extraction with 70 and 74 Per Cent Alcohol for 24 and 48 Hours

Duration of Extraction and Strength of Alcohol	New Zealand	White Club	Gold Coin	Black Don	Kahla	Adjini	Average
70% alcohol, 24 hours	1.450	1.289	1.505	1.721	1.732	1.558	1.543
70% alcohol, 48 hours	1.470	1.333	1.508	1.731	1.735	1.592	1.562
Difference	0.020	0.044	0.003	0.010	0.003	0.034	0.019
Per cent nitrogen by polariscope reading, 24 hours	.50	.53	.50	.56	.54	.57	.533
Per cent nitrogen by polariscope reading, 48 hours	.51	.53	.50	.56	.51	.54	.525
74% alcohol, 24 hours	1.270	1.086	1.293	1.320	1.446	1.278	1.282
74% alcohol, 48 hours	1.278	1.106	1.291	1.329	1.448	1.289	1.290
Difference	0.008	0.020	0.002	0.009	0.002	0.011	0.008

These results show that there is a small quantity of protein which passes into the solution even after the expiration of 24 hours contact with the solvent. This increase is marked only in

the case of the White Club and Adjini. With the rest of the samples the increase after 24 hours is very small. That this increase is not due to evaporation is shown by the fact that the bottles containing the solution weighed the same at the beginning and end of the extraction period.

Examining the work which has been previously done on this subject we find that Hummel[70] reports the amount extracted after the lapse of 44 hours to be slightly greater than that extracted at the end of 24 hours. Chamberlain[10] found the same to be true. He also compared the amount extracted at the end of 48 and 72 hours and found a small increase, but it was not as great as in the preceding 24 hours. It is possible that the extra amount extracted after 24 hours is glutenin or some nitrogenous substance of the flour other than gliadin. However, the small difference in the chemical composition of gliadin and glutenin makes this rather difficult to determine.

That the specific rotation of the gliadin is not changed to any great extent by contact with 70 per cent alcohol for 48 hours, seems probable from the results given, as the per cent nitrogen divided by polariscope reading is nearly constant for both periods. From these results it would appear that where as great accuracy as possible with our present methods is desired, in the determination of the alcohol soluble proteins, the extraction should be continued for 48 hours. But it must be borne in mind that when the length of the extraction period is increased, the error resulting from evaporation may be also increased if special precautions are not taken to prevent evaporation, by the selection of bottles with well ground stoppers and the avoiding of excessive temperature during extraction. Nor should the flour be left in contact with the alcohol for too long a time for if this be the case, part of the gliadin may become insoluble.

Influence of Strength of Alcohol on Gliadin Extracted

Considerable work has been done to determine the influence of different strengths of alcohol on the extraction of gliadin from flour, but in so far as I am aware, it has not been considered in connection with the rotation of the extract. And it is primarily, in this connection, that it is to be considered in

this article. Examining some of the work which has been previously done, on the extraction of gliadin from flour by varying strengths of alcohol, we find greatest amounts of nitrogen obtained with comparatively dilute alcohol. As for example, Snyder[70] determined the nitrogen extracted by alcohol varying in strength from 60 to 72 per cent, and found that the greatest amount 0.85 per cent was obtained with 60 per cent alcohol, and that the amount extracted decreased as the alcohol became more concentrated; the 72 per cent alcohol extracting only 0.67 per cent nitrogen. Hummel[70] determined the amount extracted with alcohol varying in strength from 71 to 81 per cent by weight and found that 70 per cent alcohol had extracted .96 per cent nitrogen and there was a gradual decrease in the yield to 75 per cent alcohol which extracted 0.66 per cent nitrogen, while the 81 per cent alcohol was found to extract only one-third as much as the 70 per cent. Shutt[70] tested alcohol ranging in concentration from 60 to 86.4 per cent by weight with similar results. While there is a greater amount of nitrogenous material extracted from the flour with the more dilute alcohol, there are facts which tend to show that the dilute alcohol also extracts a comparatively greater amount of non-gliadin nitrogen. This, together with the fact that gliadin reaches its maximum solubility in 70 per cent alcohol has led a great number, though by no means all, investigators to adopt this as the proper strength of alcohol to use in gliadin determinations, a strength which appears from the following work to be too low.

The six different flours used in this work have been tested as to nitrogen extracted by varying concentrations of alcohol together with the influence on the polariscope reading as obtained from the alcoholic extract. This was determined by extracting 7.985 grams of each of the flours for 48 hours, with 100 c.c. of alcohol varying in strength from 60 to 80 per cent, by volume. The results for the per cent nitrogen are given in Table IV.

Table IV.—The Per Cent Nitrogen Extracted From Flours From Different Wheats by Varying Strengths of Alcohol

Strength of alcohol (Per cent. by volume)	New Zealand	White Club	Gold Coin	Black Don	Kahla	Adjini	Average
60 per cent	1.487	1.324	1.459	1.617	1.757	1.602	1.541
Greater + less — than by 70% alcohol	0.017	—0.009	—0.049	—0.114	0.082	0.010	—0.063
65 per cent	1.429	1.267	1.467	1.644	1.666	1.499	1.496
Less than by 70% alcohol	0.061	0.066	0.041	0.087	0.009	0.093	0.059
70 per cent	1.470	1.333	1.508	1.731	1.675	1.592	1.551
72.5 per cent	1.254	1.093	1.296	1.484	1.480	1.371	1.330
Less than by 70%	0.261	0.240	0.212	0.247	0.195	0.221	0.222
74 per cent	1.278	1.106	1.291	1.329	1.448	1.289	1.290
Less than by 70%	0.192	0.227	0.217	0.402	0.227	0.303	0.261
75 per cent	1.270	1.116	1.282	1.326	1.447	1.287	1.288
Less than by 70%	0.200	0.217	0.226	0.405	0.228	0.305	0.263
80 per cent	1.118	0.997	1.192	1.288	1.441	1.249	1.214
Less than by 70%	0.352	0.336	0.316	0.443	0.234	0.343	0.337

The maximum amount, as may be seen, was extracted by 70 per cent alcohol, and with the exceptions of Black Don, in which there is a large decrease, and Kahla a large increase, there is a gradual decrease to 60 per cent alcohol. As the strength of the alcohol is increased from 70 to 72.5 per cent there is a large decrease in the nitrogenous extract being as an average of these determinations 0.222 per cent nitrogen. As the strength of the alcohol is increased from 72.5 to 80 per cent there is a decrease in the nitrogen obtained, but not in so marked a degree as between the 70 and 72.5 per cent alcohol. The proportion of the nitrogenous substances extracted by different strengths of alcohol, in some cases, appears to vary with the flour, but in the main they all follow the same trend.

In the light of the above results it is of interest to consider the strengths of alcohol used by a few analysts in determining the gliadin content of flour. Snyder[68] used 70 per cent alcohol. Teller[74] used alcohol having a specific gravity of .9 corresponding to about 65 per cent alcohol by volume. The same strength was used by Guess.[23] While Hamann[26] used 70 per cent alcohol containing 1 per cent acetic acid. Considering these facts it is not surprising to find Fleurent[20] stating that good flour should contain gliadin and glutenin in the ratio of 75 to 25. Snyder[71] 60 to 40, Hamann[26] 64 to 36, while

according to Kosutany[35] they vary between 76 to 24 and 68 to 32.

One fact does appear from this consideration and that is, if gliadin determinations are to have any value in determining the quality of wheat flours, a definite strength of alcohol must be used by all investigators.

The polariscope reading of solutions prepared by extracting flour with alcohol varying in strength from 60 to 80 per cent by volume was determined and is given in Table VI in the form of per cent nitrogen divided by the polariscope reading.

TABLE VI.—THE FACTOR OF PER CENT NITROGEN AS DETERMINED BY THE KJELDAHL METHOD, DIVIDED BY THE POLARISCOPE READING FOR VARYING CONCENTRATIONS OF ALCOHOL

Strength of alcohol (Per cent. by volume)	New Zealand	White Club	Gold Coin	Black Don	Kahla	Adjini	Average
60% alcohol	.53	.47	.49	.51	.58	.52	.516
Greater + less — than by 70% alcohol	.02	—.06	—.01	—.05	.07	—.02	—.009
65% alcohol	.46	.48	.49	.50	.48	.48	.482
Less than by 70% alcohol	.05	.05	.01	.06	.03	.06	.043
70% alcohol	.51	.53	.50	.56	.51	.54	.525
72.5% alcohol	.47	.44	.45	.51	.51	.48	.477
Less than by 70% alcohol	.04	.09	.05	.05	.00	.06	.048
74% alcohol	.47	.45	.46	.47	.47	.47	.465
Less than by 70% alcohol	.04	.08	.04	.09	.04	.07	.060
75% alcohol	.47	.45	.47	.46	.48	.48	.468
Less than by 70% alcohol	.04	.08	.03	.10	.03	.06	.057
80% alcohol	.44	.49	.44	.46	.52	.44	.465
Greater + less — than by 70% alcohol	—.07	—.04	—.06	—.10	+.01	—.10	—.060

An examination of the above table reveals the facts, that with few exceptions the ratio of per cent nitrogen to polariscope reading is greatest when the flour has been extracted with 70 per cent alcohol, and that this ratio decreases as the strength of alcohol increases, from 70 to 80 per cent; that the difference between the ratio as shown by the different flours, with the same strength of alcohol is least with 74 and 75 per cent alcohol. Examining the difference between the ratio as shown by the various samples extracted with 70 per cent alcohol, we find it to be .06, while with 74 per cent alcohol the difference is only .02. Comparing this later result with the difference existing

between various samples as extracted with other strengths of alcohol, except 75 per cent, we find a still greater difference. This is very important, especially where gliadin is to be determined by the polarization of the alcoholic extract and then multiplying the results thus obtained by a factor obtained from a series of determinations on different flours.

If the specific rotation for the desolved substance be calculated from the different per cents of nitrogen, and corresponding polariscope readings for the various strengths of alcohol, there are obtained some very interesting results. The calculation is made as follows: The average per cent nitrogen as given in Table IV multiplied by 5.7 gives the per cent nitrogen in terms of gliadin. These results multiplied by the weight in grams (7.985 x 91.11 per cent dry matter in flour by 100) of flour used gives the grams of nitrogenous substance in terms of gliadin contained in 100 c.c. of the solution. The average observed rotation in degrees is obtained from Table VI by dividing the average per cent nitrogen by the ratio of per cent nitrogen to polariscope reading, and multiplying this, the reading on the Sugar scale of a Schmidt and Haehsch polariscope, by 3.45.

Then applying Biot's formula $[a] = \frac{a}{l\,c}$ in which

a = observed reading,

c = weight in grams of substance calculated as gliadin in 1 c.c. of the solution and

l = length of the tube in decimeters.

We obtain as the specific rotation of the alcohol soluble substance for the different strengths of alcohol the following values: Alcohol soluble substance in 60 per cent alcohol $[a] = -80.85$, 65 per cent alcohol $[a] = -86.52$, 70 per cent alcohol $[a] = -79.49$, 72.5 per cent alcohol $[a] = -87.46$, 74 per cent alcohol $[a] = -89.80$, 75 per cent alcohol $[a] = -89.16$, and 80 per cent alcohol $[a] = -89.71$.

These results show that the specific rotation reaches its highest value when the flour has been extracted by means of 74 per cent alcohol. This fact, together with the nearly constant value obtained in the ratio of nitrogen to polariscope reading with 74 per cent alcohol points very strongly to the conclusion that

this strength of alcohol extracts more nearly pure gliadin than does any of the other strengths tested. However, the great difference in the amount of nitrogenous material extracted by 70 and 74 per cent alcohol raises the question as to whether the extraction of gliadin is as complete with 74 per cent as it is with 70 per cent alcohol. In order to throw some light upon this subject, the solubility of gliadin was determined in 70 and 74 per cent alcohol. The gliadin[57] used in the determination was prepared in the following manner.

Gluten was prepared by kneeding dough made from wheat flour in a stream of cold water until all the starch had been washed out, it was then partially dried and a moisture determination made. The moist gluten thus obtained was finely chopped and together with twenty times its weight of alcohol of such a strength that with the water in the gluten it formed an alcoholic solution containing 70 per cent alcohol by volume, was placed in a bottle. This was allowed to stand with frequent shaking for forty-eight hours. After the solution had settled for ten hours, the alcohol was siphoned off and filtered through large asbestos filters until perfectly clear. The filtrate was evaporated, under about one-half atmospheric pressure, until frothing prevented further concentration. It was then cooled and very slowly poured with constant stirring into about one hundred times its volume of ice cold distilled water, containing 5 grams of sodium chloride per liter. The gummy mass which usually collects on the stirring rod was dissolved in the least possible amount of absolute alcohol, and then evaporated under reduced pressure to a thick syrup, cooled and poured in a very fine stream with constant stirring into absolute alcohol. This precipitated gliadin was taken up with 70 per cent alcohol and again digested under reduced pressure, with the occasional addition of absolute alcohol until a thick syrup was obtained as before. The gliadin was precipitated from this as before, washed three times with "absolute" ether, and then dried over sulphuric acid.

The solubility in 70 per cent alcohol of this gliadin was found to be .0601, while in 74 per cent alcohol it was .0538. The difference in the solubility in the two concentrations is very small,

so it would appear as if just as much gliadin would be extracted from 7.985 grams of flour with 100 c.c. of 74 per cent alcohol as with the same amount of 70 per cent alcohol, for the gliadin in this weight of flour would not exceed .8 gram, which is only one-seventh of the amount 100 c.c. of 74 per cent alcohol is capable of dissolving.

With methods based on the determination of gliadin with alcohol as a solvent we must try and find the strength of alcohol in which the non-gliadin material extracted reaches a minimum and the alcohol still extracts all of the gliadin. Quite definite conclusions can be drawn on this subject from a consideration of the results obtained for the specific rotation of the alcohol extracted protein, together with the solubility of gliadin. The specific rotation of the alcohol extracted protein from wheat flour reaches its maximum in 74 per cent alcohol, which indicates that the non-gliadin material reaches its minimum value in alcohol of this concentration. 100 c.c. of 74 per cent alcohol is capable of dissolving 5.38 grams of gliadin and with the weight of flour (7.985) grams used the amount to be extracted would not exceed .8 gram. And the fact that when one-third of this amount of flour was extracted with 100 c.c. of 74 per cent alcohol, the per cent of gliadin obtained was but little greater than when the full 7.985 grams was used, would make it appear as if the conditions outlined above were most nearly reached with 74 per cent alcohol.

Infuence of Hot Extraction

Kjeldahl[31] found that the temperature of the alcohol used in the extraction of the protein from wheat meal influenced but slightly the amount extracted. While Chamberlain[9] determined the amount of protein in a sample of flour with hot and cold alcohol and obtained with the former 7.32 per cent protein and with the latter 7.47 per cent protein. The hot alcohol extracted less protein than the cold; however, it is difficult to state just what portion of this difference is due to the change in temperature of the solvent, and what is due to the change in the concentration of the alcohol during extraction and the subsequent addition of alcohol to replace that lost by evaporation.

Marion[41] apparently overcame this difficulty by extracting the flour in a closed vessel with hot alcohol. In order to test this method the six flours used in this work were extracted in closed bottles by placing 7.985 grams of each of the flours, together with 100 c.c. of 74 per cent alcohol, into tightly stoppered pressure flasks. These were weighed and then heated in a water bath at 65 degrees C. for twenty-five minutes, with occasional thorough shaking. After which they were cooled to 17 degrees C., weighed, and the nitrogen and polariscope determinations made as in the general method. The results, together with those obtained by 48 hours' cold extraction of the flours with 74 per cent alcohol, are given in Table VII. The weight of the flasks before and after extraction were practically the same, thus showing no appreciable amount of alcohol had been lost by evaporation.

TABLE VII.—THE PER CENT NITROGEN EXTRACTED BY HOT AND COLD 75 PER CENT ALCOHOL; ALSO THE RATIO OF PER CENT NITGROGEN TO POLARISCOPE READING IN EACH CASE

	Per Cent. Nitrogen			Ratio of Per Cent. Nitrogen to Polariscope Reading		
	74 Per Cent. Cold	74 Per Cent. Hot		74 Per Cent. Cold	74 Per Cent. Hot	
Variety	Alcohol	Alcohol	Difference	Alcohol	Alcohol	Difference
New Zealand	1.278	1.390	0.112	.47	.56	.09
White Club	1.106	1.165	0.059	.45	.52	.07
Gold Coin	1.291	1.393	0.102	.46	.59	.13
Black Don	1.329	1.632	0.303	.47	.61	.14
Kahla	1.448	1.671	0.223	.47	.60	.13
Adjini	1.289	1.494	0.205	.47	.64	.17
Average	1.290	1.457	0.167	.465	.587	.121

These results show a greater per cent of protein nitrogen in every case where the extraction has been made with hot alcohol than when it has been made with cold. This difference varies with the different flours being greatest with Black Don and least with White Club. In fact all the Durum varieties show a larger difference than do the common bread varieties. However, in the case of all the flours there is a large difference between the cold and hot extraction and amounts to, as an average of these determinations, 0.167 per cent of nitrogen. The ratio of per cent nitrogen to polariscope reading is also higher in the hot extraction and there is a lack of agreement in this ratio with the different flours when the hot alcohol is used as a solvent.

The solutions obtained by cooling and filtering after hot extraction were clear, but on standing for a few hours they became turbid and in a short time a fine precipitate settled out. This was filtered off and the filtrate allowed to stand for 24 hours, during which time there again developed a turbid solution. The fact that alcoholic solutions of gliadin and solutions made by cold extraction of flour may be kept for several days without becoming turbid indicates that the gliadin was either changed during the hot extraction, or that some substance other than gliadin was extracted with hot alcohol under these conditions, which, on standing, slowly separated out. That it is due to the latter cause is likely from the fact that gliadin solutions gave the same specific rotation after heating for twenty-five minutes at 65 degrees C. as they did before. From these facts it appears that the hot extraction of flour in a closed vessel gives results for gliadin determinations which are abnormally high.

Influence of Heating Flour Before Extraction

This was determined by heating 7.985 grams of each of the samples for sixteen hours in a steam bath at 96 degrees C. They were then extracted with 74 per cent alcohol for 48 hours and the nitrogen and polariscope determinations made as in the preceding work. The results together with those obtained with the air dry flour are given in Table VIII.

Table VIII.—The Per Cent Nitrogen Extracted by 74 Per Cent Alcohol From Dry and Air Dried Flour, Together with the Ratio of Per Cent Nitrogen to Polariscope Reading in Each Case

Variety	Per Cent. Nitrogen Extracted			Per Cent. Nitrogen by Polariscope Reading		
	From Dry Flour	From Air Dry Flour	Difference	From Dry Flour	From Air Dry Flour	Difference
New Zealand	1.118	1.278	0.160	.46	.47	.01
White Club	1.016	1.016	0.090	.44	.45	.01
Gold Coin	1.187	1.291	0.104	.43	.46	.03
Black Don	1.317	1.329	0.012	.46	.47	.01
Kahla	1.365	1.448	0.083	.48	.47	—.01
Adjini	1.225	1.289	0.064	.48	.47	—.01
Average	1.205	1.290	0.085	.459	.465	.006

As may be seen, there is a variation with the different flours; nevertheless, with the single exception of Black Don, the air dry flour on extraction yields considerable more protein than

does the flour which has been heated at 98 degrees C. for 16 hours. This difference is, as an average of all the determinations, 0.085 per cent nitrogen. This is not as great a difference as was obtained by Chamberlain,[9] who has studied the action of the alcoholic extraction of dry and air dry flour with the result that he obtained 4.58 per cent protein from a sample of dry flour and 7.32 per cent from the sample in the air dry condition.

The ratio of per cent nitrogen to polariscope reading, within experimental error, is the same under both conditions. It is likely that the portion which has been rendered insoluble is mainly albumen, as very little of the globulin would be coagulated at the temperature used in drying the flour. That it is not the gliadin is likely from the work of Mathewson, who has shown that gliadin appearently suffers no change when heated at the above temperature for sixteen hours. However, the conditions for gliadin are different when heated in the air dry flour than when the nearly pure gliadin is heated, so that too much reliance must not be put upon his work. The very nearly agreeing ratios of nitrogen to polariscope reading in the case of heated and non-heated flour show that if the gliadin be not rendered insoluble by the heating the substance which is rendered insoluble has very nearly the same specific rotation as that of gliadin. Furthermore, there was a very close agreement between duplicate determinations, a condition which would not be expected if part of the gliadin had been rendered insoluble.

Influence of Extraction with Ether and Then Alcohol

This determination was made to ascertain the effect of the ether soluble substances (such as lecithin, which contains nitrogen and is optically active) on the results obtained by the Kjeldahl and polariscope methods. The determinations were made by extracting 7.985 grams of each of the flours with "anhydrous ether" in a Soxlet extraction apparatus for eight hours, drying at a low temperature long enough to dispel all the ether, extracting for forty-eight hours with 74 per cent alcohol and determining the nitrogen and polariscope readings as in the preceding work. The results are given in Table IX, together

with those obtained by the direct extraction of air dry flour with 74 per cent alcohol.

Table IX.—The Per Cent of Nitrogen Extracted by 74 Per Cent Alcohol From Ether Extracted and Air Dry Flour; Also the Ratio of Per Cent Nitrogen to Polariscope Reading in Each Case

	Per Cent. Nitrogen Extracted			Ratio of Per Cent. Nitrogen to Polariscope Reading		
Variety	From Ether Extracted Flour	From Air Dry Flour	Difference	From Ether Extracted Flour	From Air Dry Flour	Difference
New Zealand	1.142	1.278	0.136	.45	.47	.02
White Club	.987	1.106	0.119	.47	.45	—.02
Gold Coin	1.229	1.291	0.062	.44	.46	.02
Black Don	1.323	1.329	0.006	.50	.47	—.03
Kahla	1.442	1.448	0.006	.50	.47	—.03
Adjini	1.278	1.289	0.011	.49	.47	—.02
Average	1.234	1.290	0.056	.475	.465	—.01

An examination of the above table shows that in the case of the common bread varieties of flour there is a much greater per cent of nitrogen extracted from the air dry flour than from flour which had been previously extracted with ether. However, with the flours from the Durum wheats the results are, within experimental error, the same for both extractions. The ratio of per cent nitrogen to polariscope reading shows a small difference in the two determinations, but there is no regularity in the difference. The solutions which were obtained from the ether extracted flour were nearly colorless, while some of those obtained by the direct extraction of the flour with alcohol, though clear, were quite highly colored. This color was greatest in the case of Black Don and Kahla and least with New Zealand and Gold Coin.

In order to determine to what extent the gliadin had been dissolved by the ether, two gram portions of gliadin prepared as previously stated were extracted with ether in a Soxlet extraction apparatus for four hours; the ether evaporated and the resulting residue taken up with 100 c.c. of 74 per cent alcohol. The rotation of this was practically zero. The nitrogen in the solution was determined by the Kjeldahl method and it was found to contain .9 mg. of nitrogen. The gliadin which had been used in the first extraction was again extracted with ether for four hours

and the nitrogen determined as before with the result that the ether had extracted .14 mg. of nitrogen. The results show that the gliadin is dissolved only very slowly by the ether and taken in connection with the results obtained by the extraction of the flour with ether they show that some flours contain sufficient ether soluble nitrogen carrying substance to materially affect gliadin determinations as made by the direct extraction of air dry flour with 74 per cent alcohol.

Influence of Temperature on the Polariscope Reading

Solutions were prepared as in the general method and the polariscope readings taken at different temperatures. It was found that solutions filtered at room temperature (about 17 degrees C.) on being cooled to below 10 degrees C. gave a turbid solution and for this reason the first reading was taken at 10 degrees C. and subsequent readings at 20, 30, 40, 50, and 60 degrees C. The average of all the results obtained from all the flours was found to be .15 more on the sugar scale of a Schmidt and Haensch polariscope at 10 degrees C. that at 60 degrees C. This would correspond to a difference of .003 on the sugar scale for a change of each degree in temperature. Therefore, a rise of 10 degrees C., which in ordinary work is far beyond the change in temperature that would be likely to occur is equal to .03 on the sugar scale. This agrees with the results obtained by Mathewson,[42] who found, when working with carefully purified gliadin, that when an allowance is made for the expansion of the solvent due to heat, the temperature at which the reading was made, had but little effect upon the rotation. A difference of 10 degrees C. between the various solutions is greater than would be likely to occur in actual determinations and this would correspond to only .012 per cent nitrogen, which is within experimental error. For this reason it is not necessary to make a correction for the differences in temperature. However, it is best when working with the polariscope to make all readings at between 15 and 20 degrees C., for above this temperature evaporation is comparatively great and below this temperature the solution, if filtered at 15 degrees C., tends to become turbid, thus preventing an exact reading.

The Influence of Non-Protein Substances on the Polariscope Reading

Some analysts have found it necessary, when determining gliadin by means of the polariscope method, to make a correction for the sugar extracted with the gliadin, while others have found this to be unnecessary as is shown from the following. According to Snyder[68] a correction is not necessary, for in the flours examined by him he found: "The combined alcohol soluble carbohydrates and non-gliadin proteids of the alcohol solution affected the polariscope reading to only a slight extent. In a number of cases where the gliadin proteids were precipitated, the non-gliadin rotatory bodies showed a reading of less than 0.20 per cent on the sugar scale, or 0.04 per cent on the gliadin nitrogen scale." Norton,[47] on the other hand, found it necessary to make a correction for the sugars which amounted to from 0.7 to as much as 1.8 per cent on the sugar scale of a Schmidt and Haensch polariscope. The same was found to be true by Shaw,[67] who obtained high values, especially with wheat meal and the lower grades of flour. While Ladd[37] in his report of an investigation of the method recommends two polarizations, one to be made directly on the alcoholic extract, the other after the protein material has been precipitated by means of Millon's reagent. Marion,[41] however, in the polariscope method as outlined by him makes only one polarization. For he considers the amount of non-gliadin material extracted in every case to be practically constant. And if determinations be made so as to include this difference in the factor he considers the results thus obtained to be fairly accurate without further corrections. This lack of agreement between the conclusions reached by various investigators is undoubtedly due to a difference in the flours examined.

Determinations have been made on the six different flours used in this work to find out the effect of non-protein substances on the results obtained by means of the polariscope. This was done by precipitating the protein material from 50 c.c. of the solution prepared as in the preceding determinations by means of 5 c.c. of a saturated solution of mercuric nitrate and then polarizing. The correction thus obtained, in terms of per cent

on the sugar scale of a Schmidt and Haensch polariscope, was found to be, as an average of all the flours, 0.223. The lowest reading was obtained for Gold Coin 0.159, and the highest for Adjini, 0.281. The results were found to be the same, within experimental error, when the flours had been extracted with cold 74 per cent alcohol in the air dry condition, after extraction with ether, after heating 16 hours, and when extracted with hot 74 per cent alcohol.

These results show that with flours similar to those used in this work it is necessary to make two polarizations so as to correct for non-protein material which has been extracted by the alcohol. Nor is it sufficient to make an average correction, for in the six flours used there was found a difference of .122 on the sugar scale of a Schmidt and Haensch polariscope, between Gold Coin and Adjini.

It is interesting to compare the average results obtained for per cent nitrogen, corrected ratio of per cent nitrogen to polariscopic reading, and calculated specific rotation for the various extractions with 74 per cent alcohol. These results are given below.

	Air Dry Flour	Heated Flour	Less than Air Dry	Ether Extracted Flour	Greater + Less − than Air Dry	Hot Extracton	Greater + Less − than Air Dry
Per cent nitrogen	1.290	1.205	0.085	1.234	—0.056	1.457	0.167
Ratio of per cent nitrogen to polariscopic reading	.430	.423	0.007	.438	0.008	.536	—.106
Calculated specific rotation	—97.01	—98.61	.....	—95.30	.....	—77.86	.....

These results show that when flour is extracted with hot 74 per cent alcohol in a closed vessel a greater per cent of nitrogen is obtained than when the flour is extracted with cold alcohol of the same strength. However, an examination of the calculated specific rotations show that the hot alcohol extracts considerable non-gliadin protein. The nitrogen extracted by cold 74 per cent alcohol from flour which had been previous extracted with ether, or had been heated before extraction, is less than that extracted from the air dry flour.

The ratio of per cent nitrogen to polariscope reading is nearly the same for air dry flour, heated flour, and ether extracted

flour, but is much higher for flour extracted with hot alcohol. The specific rotation is highest for heated flour and lowest for flour extracted with hot alcohol.

SUMMARY

The concentration of solutions of the alcohol soluble proteins is decreased on filtering through the Chamberlain-Pasteur filter. This is also the case when these solutions are filtered through layers of animal charcoal or clarified by shaking with this substance and then filtering.

Solutions of alcohol soluble proteins can be filtered through carefully prepared asbestos filters and in this manner clear filtrates obtained without materially changing the concentration of the solution.

As an average of the determinations made, .05 per cent more alcohol soluble protein nitrogen was extracted when 7.985 grams of flour was treated with 100 c.c. of alcohol than when twice this amount of flour was used with the same volume of alcohol.

The extraction of the alcohol soluble proteins does not seem to be complete, especially with 70 per cent alcohol, when the proportion of flour to alcohol exceeds two grams of flour to 100 c.c. of alcohol.

The specific rotation of alcohol soluble proteins varies but little, if any, with the concentration of the solution.

Greater accuracy can be obtained in making gliadin determinations by means of the polariscope when 7.985 grams of flour are extracted with 100 c.c. of alcohol and polarized in a 200 mm. tube than when twice this amount of flour is used with the same volume of alcohol. However, with flours from some wheats the accuracy of the method can be increased still more by extracting 7.985 grams of flour with 100 c.c. of alcohol and then polarizing in a 400 mm. tube.

The amount of protein nitrogen extracted from flours varies with the strength of the alcohol used. And with the strengths tested, 60 to 80 per cent, the greatest amount was extracted by 65 per cent alcohol by volume and there was a decrease in this amount as the strength of the alcohol increased.

A consideration of the specific rotation of proteins extracted by alcohol of varying strengths shows that alcohol of 74 per cent by volume more nearly extracts pure gliadin than does alcohol of other strengths.

From a consideration of the solubility of gliadin and the amount extracted with different ratios of alcohol to flour, it appears that with a charge of 7.985 grams of flour to 100 c.c. of alcohol, 74 per cent alcohol extracts as much gliadin as does 70 per cent alcohol.

The ratio of per cent nitrogen extracted from flour by alcohol to the polariscope reading for the solution varies with the strength of alcohol.

The extraction of flour with hot 74 per cent alcohol in a closed vessel yields more protein nitrogen than does cold extraction. However, the specific rotation of the protein extracted shows it to contain considerable non-gliadin protein material.

The heating of flour before extraction with alcohol decreases the amount of protein nitrogen extracted by 74 per cent alcohol.

Some flours contain sufficient ether soluble nitrogen carrying substances to materially affect the accuracy of gliadin determinations made by the direct extraction of flour with 74 per cent alcohol.

The rotation of alcoholic extracts of flour is only slightly affected by changes in temperature and may within certain limits be disregarded in determining gliadin by means of the polariscope.

With the flours examined, it was found necessary to make a correction for the sugars in the polariscope method.

Gliadin determinations can be made rapidly by means of the polariscope and the results thus obtained are fairly accurate, but not as accurate as those obtained by the Kjeldahl method.

From the above work it would appear that the following methods have given the best results:

The Kjeldahl Method for Gliadin.—7.985 grams of flour was extracted for 48 hours with alcohol of such a strength that with the moisture in the flour it contained 74 per cent alcohol by volume. The extraction was made in 200 c.c. bottles fitted with ground glass stoppers and were occasionally shaken during the

first 24 hours and then allowed to settle for 24 hours. At the end of this time they were filtered through asbestos filters which were prepared by placing a small amount of dry shredded asbestos into the bottom of a Gooch crucible and pouring a suspension of asbestos into the crucible sufficient to form a pad about one-half inch thick. This was washed with 74 per cent alcohol and then dried. 20 c.c. of the clear filtrate obtained by filtering through these filters was measured into a Kjeldahl digestion flask, 5 c.c. of concentrated sulphuric acid added and the alcohol evaporated off. (It was found that care should be taken at this point to dispel the greater portion of the alcohol before the addition of mercury, otherwise there is considerable frothing.) To this was added 15 c.c. of concentrated sulphuric acid and .5 gram of mercuric oxide, the solution digested, and the nitrogen determined as given in the official methods of the Association of Official Agricultural Chemists.[11]

Polariscope Method.—The clear alcoholic solution obtained as in the preceding method was polarized in a 200 mm. tube. Then to 50 c.c. of the solution was added 5 c.c. of a saturated solution of mercuric nitrate; this was filtered and polarized as before. The reading thus obtained increased by one-tenth was added to the first reading and the result multiplied by .43, which gave the per cent nitrogen. With solutions which are colorless or nearly so, it may be advisable to polarize in a 400 mm. tube and multiply by .215.

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